STAINING!

THE Ziehl-Neelsen ACID FAST STAIN
ALL STAINS BEGIN WITH A PROPERLY MADE SMEAR! See Preparation of a SMEAR from Agar or a Broth...

- The Acid Fast Stain is was devised for ONE GENUS – Mycobacteria.

- This stain is a Differential Stain as it separates microbes into one large group and a small group based on whether the microbial cell wall construction has a large amount of LIPID or not...

- AND the Primary stain will stick to ANYTHING hard to stain (under heat) – Endospores?
The GENUS **MYCOBACTERIUM** contains species of microbes that cause **Tuberculosis, Leprosy**, and other fatal illnesses.

We use non-pathogenic **Mycobacterium**

**Mycobacterium** are notoriously hard to kill and hard to stain; they also grow very very slowly.

All of these characteristics are due to the very THICK LIPID LAYER in the cell wall.

**Mycobacterium** stain Gram-positive because of their THICK cell wall composed of a THICK layer of **LIPID** + a thin peptidoglycan layer.
THE ACID FAST STAIN – Process/Overview

- The ACID FAST process involves two dyes, STEAM, & several other solutions.

- **CARBOLFUCHSIN** is known as the PRIMARY STAIN and is **STEAMED**

- **METHYLCENE BLUE** is the SECONDARY STAIN

- **DECOLORIZER** is **ACID Ethanol**

- **Water** is used to wash the excess solutions off the slide
Cover the “smear circle” with a drop or 2 of the **PRIMARY STAIN** or **CARBOLFUCHSIN**

Put paper UNDER your Bunsen Burner & Pass the slide through the flame rapidly - wait - then do it again & repeat until a small amount of the drop of stain has evaporated at the edge of the bubble or drop of stain **DO NOT OVERHEAT!**

Shake off the stain; wash gently. Add 1-2 drops of **ACID ALCOHOL** on the smear. **QUICKLY** remove it by **aiming a wash of water above the circle** - Rinse lightly with the water (until the water is almost clear) – **DO NOT OVERWASH!**

Cover the “circle” with the counterstain or **METHYLENE BLUE** for 2-3 min.

Shake off the stain. **Aiming above the circle** - Rinse lightly with water (until the water is almost clear) – **DO NOT OVERWASH!**

Blot dry add oil and view under 10x and 100x...
ACID FAST Stained Microbes
CELL WALL Comparison

- **Gram positive** – typical = thick cell wall composed of peptidoglycan

- **Gram positive & Acid Fast Positive MYCOBACTERIAL Cell Wall** = thick cell wall composed of a thin peptidoglycan layer and a THICK LIPID Layer
Problem organisms!

- Mycobacteria – 1 “hot pink” ROD makes it positive; the rest of the microbes under the scope could be baby blue or the reverse! They must be HOT PINK RODS to be positive - NOT ovals and not round!

- Bacillus – the “endospore” can take the “hot pink” color and thus be mistakenly thought to be Mycobacteria – this is NEGATIVE! Sometimes the hot pink endospores can be released everywhere but remember they are OVAL not rods! The vegetative Bacillus cell will stain baby blue.

- See PROBLEM STAINS; The Stain Game!